PB85-161388

Target Organ Effects of Disinfectants and Their By-Products

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

Feb 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-161388

EPA/600/D-85/025
February 1985

TARGET ORGAN EFFECTS OF DISINFECTANTS AND THEIR BY-PRODUCTS

by

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/D-85/025	2.
3. RECIPIENT'S ACCESSION NO. PB85 161388/AS	
4. TITLE AND SUBTITLE TARGET ORGAN EFFECTS OF DISINFECTANTS AND THEIR BY-PRODUCTS	5. REPORT DATE February 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Lyman W. Condie and J. Peter Bercz	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology and Microbiology Division Health Effect Research Laboratory, ORD U.S. Environmental Protection Agency Cincinnati, OH 45268	10. PROGRAM ELEMENT NO. ABMC1A
	11. CONTRACT/GRANT NO. Inhouse
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED Book of Conference Proceedings
	14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES P.O. Lyman Condie, TOTB, TMD, HERL (CI)	

16. ABSTRACT

This paper summarizes toxicological research conducted with chlorine dioxide and with chlorinated humic acids. Chlorine dioxide exposure decreases serum thyroxide levels in monkeys by possibly altering the uptake, distribution and excretion of iodine which is utilized for thyroxine synthesis. A 90-day subchronic study was conducted with chlorinated and non-chlorinated humic acids with rats. The most significant finding was the increased incidence and severity of hematuria in the chlorinated humic acid group (1.0 g/l chlorinated humic acid).

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 22
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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INTRODUCTION

Toxicological information relevant to drinking water disinfection practices has proliferated during the past decade. The subject of two recent conferences has been the complex issues involved in evaluating the health effects of drinking water disinfectants and their reaction by-products.[1,2] Although chlorine has been the primary disinfectant employed in the United States, its widespread use has been questioned following the observation that chlorination resulted in the formation of mutagenic compounds such as trihalomethanes (THM).[3,4] Alternatives such as chlorine dioxide (ClO_2) and monochloramine were subsequently employed at numerous water treatment facilities to prevent the unwanted generation of THMs. Within the last few years, research has been conducted to evaluate the possible health hazards arising from the use of alternative disinfectants.

Research on the adverse health effects of ClO_2 focused on hematological effects reported to be associated with ClO_2 and its inorganic by-products, chlorite (ClO_2^-) and chlorate (ClO_3^-).[5] While it was demonstrated that ClO_2^- could produce methemoglobinemia, additional research indicated that ClO_2^- produced hemolytic anemia in experimental animals at exposures lower than those required to produce significant increases in methemoglobinemia. ClO_2 has also been demonstrated to exert an antithyroid effect in African Green monkeys.[6] The mechanism(s) of action of the

antithyroid effect of ClO_2 are currently being investigated by the authors.

In addition to the potential health hazards from human exposure to residual disinfectants, risks from exposure to by-products from the reactions of disinfectants with organic material present in source water must be evaluated. A myriad of substances other than THMs are thought to arise from the oxidation and chlorination of the heterogeneous organic material present in source water (primarily humic and fulvic acids). Since the majority of these contaminants have not been identified, the health risks resulting from the ingestion of these compounds remain unknown.

Concern of potential adverse health effects of disinfectant by-products has been increased by the recent scientific recognition of mutagenic activity exhibited by the uncharacterized organic compounds.[7] Direct support for the hypothesis that mutagenic chlorination by-products result from the reaction of chlorine with humic materials present in source water has been recently documented.[8,9] However, little is known about non-mutagenic target organ toxicities of chlorinated humic substances. Data is presented in this chapter which summarizes recent findings on subchronic toxicology studies with chlorinated and non-chlorinated humic acids.[10]

MATERIALS AND METHODS

In the non-human primate studies a healthy colony of C. aethiops (African Green Monkey) of 7 females and 4 males were used. Conditions of animal husbandry, details of protocol for exposure to ClO_2, and clinical testing of *in vivo* systemic response were detailed by Bercz *et al.*[6]

Radioiodine uptake by the thyroid gland was determined as follows: After mild sedation the animal was chaired, and a bolus dose of ~0.5-1.0 μCi of $Na^{131}I$ in pyrogen free saline was injected via the saphenous vein. Organ counts were measured using a 2 cm γ-scintillation crystal at 2π geometry at a fixed and reproducible distance from the laryngeal region. Efficiency of the scintillation counts were determined by using a neck phantom. At fixed intervals (5 minutes to 24 hours) organ counts were taken. Radioiodine uptake values were computed by dividing the corrected organ counts by the total administered dose.

Male Sprague-Dawley rats (250 g) were used in the rodent experiments. The conditions of husbandry and protocols for isolated organ iodination, for *in vivo* iodine transport and for metabolism of radioiodine treated feed were published by Harrington *et al.*[11] Covalently bound metabolites of radioiodinated feed were separated from rat urine by ion exchange chromatography and the amount of organically bound radioactivity was expressed as percent of the total adminintered dose.

Solutions of commercially available humic acid and chlorinated humic solutions were prepared on a monthly basis by a previously described method.[9] Conditions of husbandry, exposure and testing protocols have been described by Condie et al.[10] for the subchronic studies. Eighty rats were randomly assigned to 5 equal exposure groups during the different experiments--distilled water, non-chlorinated (non-Cl) humic acid (1.0 g/L total organic carbon, TOC), and three chlorinated (Cl) humic groups (0.1, 0.5, 1.0 g/L TOC). Experiments were conducted for 30 and 90 days.

Experimental observations were statistically evaluated by analysis of variance. When the analysis of variance indicated a dose-related effect, F-distribution was used to detect differences between treatment groups.

RESULTS

Thyroid Studies

Table I summarizes the decrease in serum thyroxine of the monkeys after a 6 week exposure to the various chlorine oxides and to chlorine. Table II provides the results of radioiodine uptake studies at the start, immediately after, and 6 months after cessation of exposure to 100 ppm ClO_2.

ClO_2 treatment of isolated rat stomachs produces an increase in binding of ^{131}I to gastric tissue (Table III). Leaching each organ in saline for an additional 24 hours did not liberate further radioactivity indicating that the residual radioactivity bound to the tissue was not dialyzable.

Administration of radioiodine after it is bound to feed nutrients by prior treatment with ClO_2 *in vitro* resulted in more pronounced differences in gastrointestinal retention of ^{131}I (Table IV). After a 24 hour digestion period, the gut retained significantly larger portions of the radioisotope in the animals receiving ClO_2 treated chow. Fecal elimination of the radioiodine was found to be significantly increased by ClO_2 treatment. Commensurate with the high fecal clearance, the amount of the ^{131}I excreted in the urine decreased in these animals and the organically bound urinary iodine was ten fold greater.

Studies with Humic Acids

All animals survived the 30-day exposure period and appeared to tolerate the drinking of chlorinated and non-chlorinated humic substances. Food and fluid consumption, weight gain, organ weights, biochemical measurements, and most clinical chemistry and hematologic parameters were similar between control and treated animals (Table IV). The most striking result of this experiment was the occurrence of hematuria in treated animals. Therefore, a 90-day subchronic study was conducted to further evaluate the relationship of chlorinated humic substance exposure and production of hematuria in Sprague-Dawley rats.

Significant results of the 90-day study are summarized as follows: The average weight gain was significantly decreased in the high chlorinated humic acid group when compared to controls. The only organ weight that was significantly larger than the

corresponding control value was the kidney weight of the 1.0 Cl humic group. There were no significant differences between groups in regard to hematological parameters. Although there were statistically significant differences in some of the clinical chemistry values, the changes were slight and were within the normal distribution range. The microscopic observations and chemical analyses of the urine collected at approximately 30 and 90 days of the experiment were unremarkable except for the occurrence of hematuria and the demonstration of renal pelvic crystals in many of the hematuric rats. Hematuria was most pronounced in the 1.0 Cl humic group at both 30 and 90 days. The incidence and severity of hematuria was greater at 90 days when compared to 30-day period.

A thorough histopathological evaluation was performed on the kidneys and urinary bladders from the rats k' ed following the 30- and 90-day studies as well as the entire urinary tract following the second 90-day subchronic exposure. A few changes, such as chronic inflammation and epithelial hyperplasia, were observed in the urinary bladders of these rats; however, these changes were not considered unusual for Sprague-Dawley rats and were interpreted as spontaneous incidential lesions. Renal changes including nephropathy, interstitial inflammation, and pyelonephritis were observed in each group but did not appear to be treatment-related. The cause of the hematuria could not be

elucidated by the histopathological examination but may be related to renal crystal formation.

DISCUSSION

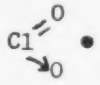
Statistical analysis of each monkey's serum thyroxine levels following exposure to various disinfectants indicated that only ClO_2 affected thyroid hormone homeostasis. The lack of thyroid effects of the other chlorine oxides, including that of perchlorate (a known thyroid suppressant), indicates that chlorine dioxide is a unique inhibitor of thyroid metabolism.

Thyroid suppression by perchlorate salts is known to occur via competitive inhibition of iodine uptake into the thyroid gland[12,13] which requires a certain level of saturation by perchlorate. Since we have demonstrated that ClO_2 is rapidly destroyed in contact with saliva and with stomach contents (over 98% reduced to chloride within seconds)[6], one may conclude that the effect of ClO_2 on the thyroid gland is neither due to it's absorption nor to the absorption of reduction products which could subsequently inhibit iodine uptake in an manner analogous to perchlorate.

The near doubling of radioiodine uptake at the end of subchronic exposure implies that the monkeys developed a degree of iodine deficiency during the subchronic ingestion of ClO_2. The effect, however, shows clear signs of reverting back to normal after cessation of exposure. Based on the data presented here

and previously[11] it is possible to postulate a plausible mechanism by which iodine deficiency could develop.

Chlorine dioxide is a very potent oxidizing species, possessing a free radical structure,



it's redox potential (1.9 V) being the highest among the chlorine oxides and is about 1 V above that of hypochlorite. Due to it's exaggerated chemical potential this disinfectant reacts in the alimentary canal with inorganic iodide of nutritional origin. The elemental iodine in turn reacts with organic molecules present in saliva and with the stomach contents. A few plausible organic substances which could covalently add iodine across reactive double bonds or in complex poly-alcohol matrices are:

Tyrosine Containing Proteins	$\xrightarrow[ClO_2]{I^-}$	Ring Iodinated Tyrosine Residues
Polyunsaturated Fatty acids	$\xrightarrow[ClO_2]{I^-}$	Polyiodinated Long Chain Carboxylic Acids
Starches and Cellulose	$\xrightarrow[ClO_2]{I^-}$	Iodinated and Complexed Molecules

Since iodide (I^-) is the only form of iodine which can be utilized for thyroxine synthesis[14], a steady state chronic shift of the inorganic iodide pool into covalently bound forms could establish a bona fide iodine deficiency, even though many of the iodinated molecules may be absorbed.

In fact, our experiments with isolated rat stomachs (Table III), with in vivo administration of radio iodine and ClO_2[11], and in vivo metabolism of in vitro radioiodinated nutrients (Table IV) provides support for this hypothesis. These studies have demonstrated that when chlorine dioxide and radioiodine are instilled into the stomach in a single acute dose, the radiolabel becomes partly attached to the gastrointestinal wall and contents. When ClO_2, radioiodine and feed are pre-mixed and then administered to rats, a very sizable portion of the radiolabel is lost via the feces unabsorbed. Furthermore, a significant portion (about 2%) of the urinary iodine appears as organically bound.

In the evaluation of the health impact of ClO_2, the potential for in vivo formation of iodinated organics should be a focus of concern. This could be an analogous to the in vivo formation of chlorinated organics during oral ingestion of hypochlorite disinfected water.[15] Our present hypothesis is that some of these iodinated compounds may be thyroid suppressors acting by enzymatic or feedback inhibition on T_4 synthesis. Thus the endocrinological effect of ClO_2 may be due to a combination of several factors. Since the other disinfectants are themselves oxidizing agents, it is possible that they also give rise to similar iodination reactions, albeit to a much lesser extent. Currently the question of in vivo formation of iodinated organics, their chemical and toxicological characterization, as well as measurment of

iodide deficiency are under detailed investigation in our laboratory.

It is not surprising that drinking water disinfectants can react with humic material to produce a diverse group of by-products. A key issue that HERL is currently addressing is whether chemicals produced through disinfection of drinking water have significant biological activity. Our laboratory has published research findings that the chlorination of humic substance yields mutagenic by-products.[8,9] Since human health risk assessment cannot be soley estimated by in vitro test results, subchronic exposures were conducted with chlorinated and non-chlorinated humic acid solutions.

The most significant finding of these studies was the increased incidence and severity of hematuria in the 1.0 Cl humic group. Increased kidney weight in the 1.0 Cl humic group may represent a response to a subchronic insult, possibly from irritation from renal crystals. Microscopic examination of freshly removed kidneys from the 1.0 Cl humic rats revealed the presence of numerous crystalline bodies in the renal pelvis. Unfortunately, the quantity of these materials isolated was insufficient for IR spectrosocpy. Perhaps the renal crystals from chlorinated humic acids are accelerating idiopathic renal changes.

This study was designed to determine if chlorination of humic acid material, which is found in finished drinking water, presents a health problem. The highest no observable effect

level in this study was 500 mg/L TOC which represents a safety factor to the human population of least 100 fold since 95% of the municipal drinking water systems tested in a national survey have TOC levels less than 5 mg/L. Other studies are currently underway at HERL to better understand the relative hazards of drinking water chlorination and its various alternatives (i.e., chlorine dioxide, chloramines, ozone).

ACKNOWLEDGMENTS

We wish to thank Mr. Dana Laurie, Mr. Robert Harrington, Mr. John Glass, Ms. Carolyn Smallwood, and Ms. Lillian Jones for their excellent assistance in this research and Ms. Patricia Underwood for accurately typing the manuscript.

This article has been reviewed by HERL, U.S. Environmental Protection Agency and approved for publication.

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Table I. Effect of Chlorine Dioxide, Chlorine, and Monochloramine in Drinking Water on Serum Thyroxine Levels of Non-Human Primates[a]

Chemical	Drinking Water Concentration	Equivalent Dose	Average Shift from Control Values % (n=12)[b]	
			7 Days	42 Days
ClO_2	30 ppm	3 mg/kg/day	-	+ 4
ClO_2	100 ppm	9 mg/kg/day	-6	-26[c]
NH_2Cl	100 ppm	10 mg/kg/day	-	+ 9
Cl_2	125 ppm	10 mg/kd/day	+3	- 1
$NaClO_2$	400 ppm	58 mg/kg/day	-	-11
$NaClO_3$	400 ppm	54 mg/kg/day	-	+ 8

[a]African green monkey (Cercopithecus Aethiops).
[b]Range of control group means of serum thyroxine levels was 4.7-5.3 µg/dl.
[c]Significant decrease $p < 0.05$.

Table II. 24 Hour ^{131}I Thyroid Gland Uptake in Female Monkeys (n=11)

	Mean Uptake % ± SEM
Pre-exposure (Over 1 year tap water)	9.15 ± 1.14
90 Days 100 ppm ClO_2 in drinking water	15.48 ± 1.32[a]
90 Days Recovery Period	10.23 ± 0.57

[a]Significant increase $p < 0.01$.

Table III. Effect of ClO_2 on Iodine Binding to Whole Isolated Stomach[a]

% of Dose of ^{131} Bound/g Stomach	
Controls (n=5)	ClO_2 Treated (n=5)
0.32 ± 0.04	2.10 ± 0.22[b]

[a]Final concentration of ClO_2 in stomachs is about 15 ppm.
[b]Data are expressed as mean values ± SEM and are analyzed by Student's t-Test. Significant increase $p < 0.01$.

Table IV. Gastrointestinal Distribution and Elimination Pattern of ^{131}I From High Protein Chow Which Had Been Exposed to ClO_2 *In Vitro*[a]

	Stomach & Conterts	Intestine & Contents	Colon & Contents	Thyroid Gland
Feed + ^{131}I	1.6 ± 0.3	0.76 ± 0.11	0.69 ± 0.09	14.0 ± 2.3
Feed + ^{131}I & ClO_2	1.1 ± 0.2	1.2 ± 0.2[b]	2.3 ± 0.5[b]	10.5 ± 1.6

	24 Hour Fecal Collection	24 Hour Urine Collection	Amount of Organically Bound ^{131}I in Urine
Feed + ^{131}I	1.9 ± 0.6	44.4 ± 4.1	0.24 ± 0.05
Feed + ^{131}I & ClO_2	17.4 ± 1.1[b]	34.8 ± 2.7	2.2 ± 0.4[b]

[a]Each value represents the mean ± SEM of the percent of total administered ^{131}I that was retained per organ or that was excreted in 24 hours (n=11).
[b]Significant increase $p < 0.001$.

Table V. Summary of Negative Observations Following 30-Day Exposure to Chlorinated and Non-chlorinated Humic Substances

Body Weights	Clinical Chemistry
Organ Weights	Creatinine
Organ/Body Weights	Albumin
	Protein
	SGPT
	BUN
	LDH
	SGOT
	Biochemical Measurements
Food Consumption	Hepatic Microsomal Cytochrome P-450
Fluid Intake	Renal Slice Uptake of P-aminohippurate
Hematology	Urinalysis
WBC	PH
MCV	Glucose
HCT	Ketones
HB	Bilirubin
	Urobilinogen